GENOTYPIC DIFFERENCE IN RESPONSE TO HEAT SHOCK TREATMENT IN SOME TOMATO CULTIVARS GROWN UNDER COLD STRESS

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Tomato (*Lycopersicon esculentum* L.) belongs to the family *Solanaceae* which is considered one of widely consumable nutritious and widely grown vegetable in the world. Tomato is one of the most important vegetable crops in the whole world after potato both in area and production (Shoaib *et al*., 2012). It is extensively cultivated in the tropical and subtropical regions of the world all year round. However, their production is seriously limited because of abiotic stress such as drought or salinity and extreme temperature (Cuartero *et al*., 2006). The requirements for high yield and premium quality are represented in a relatively cool, dry climate. However, it is adapted to a wide range of climatic conditions from temperate to hot and humid tropical. The optimum temperature for most varieties ranged between 21 and 24°C. The minimum, optimum and maximum temperature for all development stages ranges from 11-18, 16-29 and 20-24, respectively (Naika *et al*., 2005).

Rate of plant growth and its development stages is depending on the surrounding temperature and each cultivar has a specific minimum, maximum and optimum temperature (Hatfield and Prueger, 2015). Low temperature or cold stress is an environmental factor that effect on plant growth and crop productivity and leads to substantial crop losses (Hasanuzzaman *et al*., 2013). Ntatsi *et al*. (2014) reported that tomato fruit yield become lower at low temperature. Such abiotic stress may cause protein disfunction, and many physiological effects.

Egyptian tomato cultivars are preferring the warm growing season, forest free, and the optimum temperature ranged from 18°C to 29°C, while low temperature of 13°C at the night leads to the death of most of the pollen and stop fruit set (Rashwan, 2016). As well, Abou-Shleel and El-Shirbeny (2014) mentioned that the critical factor in tomato fruit setting is the night temperature. Based on the climatic recorded data in Egypt for the last four previous years; the difference between day and night temperatures is in increase and the lowest recorded tempera-

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ture at the night was in the average of 7°C. At this low temperature, tomatoes production will be minimized. This study aims to investigate the effect of heat pre-treatment on tomato tolerant to cold stress (7°C) according to the productive traits of some tomato genotypes cultivated in Egypt. Moreover, gene expression response analysis of some heat tolerant a associated genes under our experiment condition was carried out.

MATERIALS AND METHODS

Four of the common cultivated tomato cultivars in Egypt, i.e., Agyad16 (AG), Typhoon (TY), Carmen (CAR) and Hybrid super strain b (HB) were selected in this study. These cultivars were obtained from Horticulture Research Institute (Agyad16), the cultivar Typhoon from Agrimatco (Agriculture Company in Egypt) and Carmen and Hybrid super strain b from El-Shlaqany plantation at Kafr Elsheikh.

Laboratory and nursery pretreatments experiments

To study the heat pretreatment on tomato cold tolerant; our devised pre-treatments, i.e., cooling, heating and control were tested on the four selected tomato genotypes. Overnight soaked tomato seeds were pretreated with heating 45±2°C for 90 min. or cooling 3±2°C for 180 min. comparing with control incubated at room temperature (25°C). Seeds were then planted in peat-moss individually and incubated in greenhouse till germination and growing up to 32 days old. Seedlings at 32 days were then grown at 7±2°C in growth chamber comparing with control incubated at 16-23°C.

The pre-treatments were run in triplicates, 25 seeds in each replicate. After subjecting to the pretreatments, replicates in flat trays filled with a standard peat-moss were transferred to the greenhouse and irrigated daily for 32 days at the faculty of agriculture, Tanta University, Egypt. Seedlings of each pretreatment were incubated at 7±2°C (average of minimum winter temperature for 2012 to 2015) (Central laboratory for agricultural climate) for 21 days at 11/13 hour day/night.

Morphological treats

Shoot and root length (cm), leaf area (cm^2), fresh and dry weight (g $plant^{-1}$) were measured at 53 day old according to (Khan *et al*., 2016). Shoot length (cm) was measured from substrate medium surface to the vegetative point. Leaf area (cm^2), the leaf width (W) and length (L) of the leaves sampled were measured by a simple ruler. The leaf area calculated by this equation ($LA = 0.5 \times L \times W$). Fresh weight was estimated by weighting the whole plant. Dry weight (g), sample of each cultivar was dried in the oven at 105°C for 3 hours and weighted to get dry matter (g).

RT-PCR analysis

Real-time PCR has become the method of choice to measure accurately

transcript abundance of selected genes (Gachon *et al*., 2004).

The q RT PCR performed using c-DNA, synthesized by using Promrga kit. Whereby 2.5 µl (5x) buffer with $MgCl_2$, 2.5 µl (2.5 mM) dNTPs, 1 µl (10 pmol) oligo dT primer, 2.5 µl RNA (2 mg/ml), 0.5 unit reverse transcriptase and Dd H_2O up to 25 µl. PCR amplification was performed in a thermal cycler programmed at 42°C for 1 hr, 72°C for 10 min (enzyme killing) and the c-DNA product was stored at 4°C.

The q RT PCR performed in final reaction volume of 20 µl by adding: 0.5 µl (10 pmol) of the forward primer, 0.5 µl reverse primer, 4 µl (5X) EVA green Master mix, 1 µl c-DNA for each sample and Dd H_2O up to 20 µl.

Five primers (Table 1) for the selected genes associated with stress tolerance were designed based on sequencing data of expressed sequence tags (ESTs) data base. Primers were ordered from Bio search Technologies Company.

Productive traits in the open field

All tomato seedlings at 53 days (under stress and control) had been transferred to open field and were irrigated and fertilized every 14 days. All yield data were measured and calculated during total production season.

Fruit number/plant: Ten plants per cultivar were randomly chosed for calculate the average of fruits number per plant.

Fruit weight: The fruits of 10 plants (of each cultivar/treatment) during fruiting period were weighted and the average weight of fruits was calculated.

Statistical analysis

The obtained data were analyzed using the statistical software SAS 9.1, one way and multi-factorial (ANOVA) was used to determine differences between both of pretreatments and treatments. Mean separation was done by Duncan test.

RESULTS AND DISCUSSION

Morphological traits

Fresh and dry weight; shoot and root length; leaves and branch numbers; and leaf area, of the pretreated tomato genotypes (heating/cooling) and grew at 16-23°C were varied comparing with the control plants as shown in Table (2).

Plant fresh weight of the heat pretreated seeds, was significantly decreased. The cultivar AG recorded 3.1 g and CAR 2.93 g, while it increased by cooling pretreatment in CAR (8.2 g), HB (4.5 g) and TY (5.5) as shown in Table (2). Additionally, dry weight was significantly decreased than control under heating in AG (0.34 g) and HB (0.32 g), however, it was significantly increased than control under cooling in CAR (0.91 g) (Table 2). The results of this study (Table 2) revealed that shoot length was significantly increased under heating and cooling in most examined cultivars, however, it was in-

significantly affected by heating or cooling in AG and decreases significantly than control under heating in HB (12 cm). In root length, there was a significantly decreasing than control in CAR (7.4 cm and 10 cm) under heating and cooling respectively and in HB (9.2 cm) under cooling, while there was increasing than in TY (12.8 cm) under cooling. Leaves number was significantly increased than control in CAR (19) and decreased in HB (16.7) under heating; on the other hand, it was significantly than control in all examined cultivars under cooling except AG. Leaf area was significantly decreased than control under heating in CAR (3.8 cm^2), HB (4.2 cm^2) and under cooling in HB (5.6 cm^2) also. However, branch numbers were insignificantly affected by heating or cooling in all tested cultivars (Table 2).

Pretreatments (heating/cooling) followed by 7°C stress

The measured morphological treats (fresh and dry weight; shoot and root length; leaves and branch numbers; and leaf area shown in Table (2) of the pre-treated tomato genotypes (heating/cooling) flowed by grew under cold stress (at 7±2°C) were varied Fresh weight was significantly decreased under stress in two cases (with and without pre-treatment). It became lower than control in CAR, AG with and without heat and cool pre-treatment. The same result was obtained for stressed cultivars HB, VT for cool pre-treatment and HB under stress only compared to control. On the other hand fresh weight was decreased than stress only in some cultivars and increased in others; however the difference was insignificant under heating and cooling pre-treatment.

Dry weight was significantly decreased under stress in all examined cultivars with and without pre-treatment cooling and heating compared to control. But when compared to stress only; dry weight almost was insignificantly affected by pre-treatments (Table 2). Shoot and root length were significantly decreased in stressed cultivars HB, VT and AG with and without pre-treatment compared to control. The same result for shoot and root length was obtained for stressed cultivar CAR with cool pre-treatment and CAR under stress only for root length compared to control. Shoot length under heating increased significantly than stress only in CAR and TY, it recorded 12.1 and 10.3 cm in both cultivars, respectively, whereas under cooling it decreased significantly than stress only in AG (7.3 cm) and CAR (8.9 cm) and increased significantly in TY (10 cm). On the other hand, root length increased significantly than stress only under heating in CAR and TY it recorded 8.5 and 8.8 cm in both cultivars, respectively, while under cooling, root length was significantly increased than stress only in CAR it was recorded 11.3 cm (Table 2). The results in Table (2) showed also that, leaves number were significantly increased than control under stress in CAR for heat and cool pre-treatment, HB, VT and AG just for cool pre-treatment. But the opposite true in stressed cultivar VT with heat pre-

treatment; it became lower than control. Leaves number is insignificantly decreased than stress only under heating in TY (10.7), while it decreased significantly under cooling than stress only in AG (10.7) and CAR (12.3), while the opposite was true in HB, where it increased significantly (12.7) than stress only (10) under cooling. Singh *et al*. (2012) stated that chilling stress reduced the plant height, fresh and dry biomass of maize seedling. Branch number was insignificantly affected with stress only and with pre-treatment compared to control or stress only.

Leaf area was significantly decreased in stressed cultivars VT for heat and cool pre-treatment and AG for heat pre-treatment compared to control. Leaf area was insignificantly affected than stress only in all cultivars under both of cooling and heating except in CAR it was significantly higher than stress only under heating. However, Abd-Elmageed *et al*. (2003) found that fresh and dry weight, leaf area and plant height was decreased under heat shock. As well, they reported that exposure plants to heat stress decreased the stem growth resulting in decreased plant height. Our results indicated also that, branch numbers were insignificantly affected under all conditions and in all examined cultivars (Table 2). Melton and Dufault (1991) found that leaves number, leaf area and branch number were decreased under stress. Decreasing fresh and dry weight, shoot and root length under stress in some investigated cultivars is in accordance also with Wang *et al*. (2003) who were reported that change in temperature leads to a series of morphological and physiological changes. Additionally, Hasanuzzaman *et al*. (2013) mentioned that decreasing in leaves number, leaf area and branch number under heat and cool treatment can be attributed to alteration in cell division and cell elongation rates. Our results indicated that utilizing of heating as pre-treatment led to enhancing morphological parameters under stress condition (low temperature) especially in CAR and TY. Which mean that heating pre-treatment may decline the effect of stress in this cultivar. In this context, Sabehat *et al*. (1998) reported that exposing plants to moderately high temperatures for short periods often induces thermo tolerance, which allows them to survive under higher, normally lethal temperatures. They also stated that the existence of cross-tolerance, such as plant exposure to moderate stress conditions induces tolerance to other stresses. For example, high temperature stress has a positive relationship to chilling injury in a number of fruits and vegetables, such as avocado, cucumber, pepper, and tomato. Therefore, this board range of plants that are showing this cross-tolerance suggests that it may be a general response.

Singh *et al*. (2012) also found a positive correlation in their study between growth characters and morpho-physiological and biochemical traits. This may interpret the changes in some cultivars than others due to low temperature stress in our experiment. Sabehat *et al*. (1998) added that protection against chilling injury by high temperature treat-

ment has been found in mung bean hypocotyls and cucumber cotyledons and seeds. In addition, in their study loss of protection was correlated with the disappearance of heat shock protein from the tissue. Sabehat *et al*. (1998) also conducted a correlation between the expression of some genes and the acquisition of tolerance to low temperatures. Such a correlation suggests an involvement in a protective mechanism against chilling injury. As well, heat-shock genes that are involved in this process may be used for molecular breeding to generate low- and high-temperature-resistant transgenic plants such as tomato in our climate conditions.

Fruit numbers

The pretreated (heating/cooling) followed by growing under cold stress condition, fruit numbers became lower than control in the cultivars HB and TY. While the cultivar AG showed increase fruit number. The cultivar CAR showed the higher fruit numbers under cold stress with only heat pre-treatment (Fig. 1). Fruit numbers were decreased than stress only under cooling pre-treatment in all examined cultivars except AG, while they were increased than stress only in all examined cultivars except HB under heating pre-treatment. This means that heating pre-treatment led to reducing the stress effect and simulated productivity in all examined cultivars except on HB. As well, cooling led to the same thing in AG under stress condition. The increasing of fruit number in AG under cooling pre-treatment is not in accordance with (Ntatsi *et al*., 2014) they reported that fruit yield reduced when tomato exposed to low temperature. In addition, decreasing of other examined cultivars at low temperature is not accordance with (Ploeg and Heuvelink, 2005) and (Adams *et al*., 2001). Reduction in fruit numbers due to low temperature often lead to flower abortion, pollen and ovule infertility, breakdown of fertilization, poor seed filling, decreases in seed setting which eventually reduce the grain yield (Hasanuzzaman *et al*., 2013). Under heat and cool pre-treatment fruit number was increased in AG which means that pre-treatments decreased the stress but the opposite true in HB. On the other hand, fruit numbers decreased than control under cooling pre-treatment in non-stress conditions in TY and HB only, while there was a similar trend of heating pre-treatment such as in stress conditions. Therefore, our results, in terms of fruit numbers, indicated that under stress conditions (low temperature), if heating or cooling was used as pre-treatment, AG considers the most tolerance examined cultivars to low temperature. On the other hand, if heating was used as pre-treatment, all examined cultivars except HB are tolerant to low temperature and the highest tolerance one is CAR.

Fruit weight

In stress conditions, fruit weight followed similar trend to some extent such as fruit numbers; it was decreased than control in HB, TY for cool pre-treatment and TY for heat pre-treatment

(Fig. 2). While, cultivars HB, AG for heat pre-treatment and AG for cool pr-treatment became higher than control. But when fruit weight compared to stress only; it became lower under cooling pre-treatment in all examined cultivars except AG, while they were increased than control in all examined cultivars except TY under heating pre-treatment. Decreasing fruit weight under stress (low temperature) this in accordance with (Heuvelink, 2005) they reported that average fruit weight become lower under low temperature. Although, utilizing heating as pre-treatment minimizing the effect of low temperature in most examined cultivars, decreasing fruit weight under heating pre-treatment can be explained by statement of (Adams *et al.*, 2001) that elevating the temperature often result low in mean fruit weight.

The cooling pre-treatment flowed by growing at normal temperature; fruit numbers were decreased comparing with control, while heat pretreatment was useful by increasing the fruit numbers (except in HB). Our results indicated that fruit weight of the cultivar AG was the best for fruit weight.

Heat pre-treatment required low temperature tolerant in all tested cultivars specially the cultivar AG, while only the cultivar Ty was not.

Differences in gene expression

Gene expression response to our pretreatment (heat/cold) flowed by growing under cold condition was examined for the five selected genes (P5cs, BIP, Hsfa1, S13 and APX) using qRT–PCR.

Accumulation of P5cs protein in living cells under freeze-induced stress is observed for a variety of plants and animals (Szabados and Savouré, 2010). As a result, it leads to their cold tolerance (Stewart and Lee, 1974). Several reports reveal that over expression of P5CS results in an increased proline level as well as osmotolerance in transgenic plants (Vendruscolo *et al*., 2007).

Isoenzymes APX are critical components that prevent oxidative stress in photosynthetic organisms (Shigeoka *et al*., 2002).

The transcriptional regulation gene S13 was found higher in salt stress than control in tomato, this result is agree with Zhou *et al*. (2007).

The Endoplasmic Reticulum (ER) binding protein BiP is a molecular chaperone, helps newly synthesized proteins to assume their correct conformations, or associate with unrecoverable proteins to prevent them from exiting the ER until they are digested (Gething, 1999). In addition, protein disulfide isomerase (PDI), which catalyzes the formation or the rearrangement of disulfide bonds, participates in the quality control process of ER proteins (Noiva, 1999). The HsfA1a gene plays a critical role in drought stress tolerance in tomato, its positive role in the induction of autophagy under drought stress (Fu *et al.*, 2015). In tomato plants, over expression of HsfA1 gene family

resulted in heat-stress tolerance (Mishra *et al.*, 2002).

Heat pre-treatment was increase in the expression of some genes such as P5cs (1.19, 22.97 fold), BIP (1.5, 5.79 fold) and APX (34.5, 2.25 fold) in AG and HB respectively in addition to S13 (2.4) in HB genotype but the other gene of each genotype was decreased (Fig. 3). On the other hand heat pre-treatment with stress was increase in the expression of some genes such as BIP (1.14 fold) and APX (12.9 fold) in AG, P5cs (5.06 fold), BIP (2.94 fold) and S13 (1.45 fold) in HB genotype but the other gene of each genotype was decreased (Fig. 3).

Cool pre-treatment was increase in the expression of some genes such as APX (10.13 fold) in AG, P5cs (4.48 fold) and HsfAa1 (7.23 fold) in HB genotype but the other gene of each genotype was decreased. On the other hand cool pre-treatment with stress was increase in the expression of some genes such as BIP (1.43 fold), HsfAa1 (1.21 fold) and APX (38.5 fold) in AG, P5cs (3.22 fold), BIP (5.85 fold) s13 (2.6 fold) and APX (1.68 fold) in HB genotype but the other gene of each genotype was decreased (Fig. 3). Stress was increase in the expression of some genes such as P5cs (1.44, 5.74 fold), BIP (3.52, 2.93 fold), S13 (1.03, 2.05 fold) and APX (62.4, 1.17) in AG and HB genotype respectively but the other gene of each genotype was decreased.

It is recorded from our results that the expression of APX in AG cultivar was highly significant increased under stress (62.4 fold) comparing with control, but this level was higher than the pre-treatment heat and cool without stress. Interestingly, the expression of APX in the case of cool pre-treatment with stress was the highest (83.5 fold) and reflected on cold tolerance with good yield, this result meet our aim of study. On the other hand, heat pre-treatment with stress induced lower gene expression than other treatments, while the yield in this case was the best. Considering to the yield; heat pre-treatment could be our conclusion for cold tolerance with higher yield. Our demonstration for this situation may be due to the input of other substrates in the pathways resulting cold tolerant. Result for cool pre-treatment with stress means that cool pre-treatment decreased in stress effect by reducing in free radicals and oxidative stress depending on the level of APX expression (Shigeoka *et al.*, 2002). They reported that APX iso enzymes are critical components that prevent oxidative stress in photosynthetic organisms. This agrees with plant productivity (fruit eight and number) it got higher than control and stress only. But for heat pre-treatment with stress APX expression got down than stress only. Which mean that heat pre-treatment was decreased the expression of APX and increasing in free radicals and oxidative stress. But this disagrees with the productivity. So the difference of gene expression between S and H+S (S = 62.4 while H+S = 12.9) could refer to the expression of P5CS gene and others, which help in the direction of reducing of oxidative

stress. The expression of P5cs gene was increased under heat pre-treatment; it means that P5cs participate in defense mechanism via reducing oxidative stress. Its accumulation in living cells under freeze-induced stress is observed for a variety of plants and animals (Szabados and Savouré, 2010). The P5cs expression for AG agrees with plant productivity it became higher than control and stress only. Whereas in HB genotype stress only increased in P5cs and APX expression compared to control. This led to decreasing in stress and free radicals (Shigeoka *et al*., 2002). This result for HB agrees with plant productive (fruit number) it became higher than control and another treatment. On the other hand decreasing in expression of APX for HB cultivar could refer to the expression of P5cs gene and others, which help in the direction of reducing of oxidative stress. The expression of P5cs gene was increased under heat pre-treatment with stress compared to control and this led to decreasing in stress and free radical (Shigeoka *et al*., 2002). This result agrees with the productivity (fruit weight) for HB genotype it became higher than control.

Result of HsfA1a for AG genotype showed that heat and cool pre-treatment with stress led to decreasing in stress and miss folded protein (Fu *et al*., 2015). This result agrees with productivity (fruit weight and number) it became higher than control and stress only. Results for HsfA1a could refer to the decreasing in BIP expression and heat and cool pre-treatment was induced the expression of HsfA1a. Inducing HsfA1a led to reducing in miss folded protein which made the plant did not need to high expression of BIP.

The high expression of BIP, S13 and HsfA1a for HB cultivar under stress with cool pre-treatment didn't combine with high productivity (Rizhsky *et al*., 2004; Mittler 2006) reported that gene expression pattern in tobacco and Arabidopsis plants grown under a combination of drought and heat stress is different from the expression pattern observed when these stresses were applied independently. And this may back to the activity of other genes which contacted with the productivity. Morphological parameters for this cultivar under stress with cool pre-treatment were decreased significantly and this normal reaction for the high expression of heat chock proteins and chaperones. The decreasing in morphological parameters led to decreasing in the productivity which combined with late in flowering. From the last results we can tell that the high expression of APX and P5cs refer to more tolerance for cold stress and high productivity.

Finally, our results showed clear differences of heat pretreatments on the response of the studied Egyptian tomato cultivars to cold stress. Heating pretreatment induced morphological and productive traits of the examined cultivars especially AG and CAR. Therefore, the protection against chilling injury can be correlated to high temperature pretreatment which suggests an involvement of heat

shock proteins in a protective mechanism against chilling injury such as molecular breeding to generate low- and high-temperature-resistant transgenic tomato plants in Egypt climate conditions. The high expression of APX and P5cs refer to more tolerance for cold stress and high productivity.

SUMMARY

Soaked seeds of four tomato genotypes were subjected to three pretreatments; i.e. cooling (3±2°C for 180 min), heating (45±2°C for 90 min) and control (22-25°C) and then planted in peat-moss individually and incubated in greenhouse till germination and growing up to 32 days old. Seedlings were then grown at 7±2°C in growth chamber.

Our results indicated that utilizing of heating pretreatment led to enhancing morphological parameters under stress condition especially in Carmen (CAR) and Typhoon (TY) while the opposite was in CAR under non-stress conditions. In the field stage, heating increased fruit weight and fruit number in CAR followed by AG and TY. Therefore, the results indicated that CAR and AG are the most tolerant cultivars for low temperature induced by shock heating pretreatment. The high expression of APX and P5cs refer to more tolerance for cold stress and high productivity.

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Table (1): Primer name, sequences and annealing temperature.

Primer code	Gene	Accession Number	Primer sequence 5'……3'	Product length	Annealing Temperature °C
Am1	BIP	XM_004234937.2	F (GAAGCACTTGAATGGTTGGACG) R (GCCGTGATAACTGGGTTGCA)	101	55
Am2	actin	XM_004235020.2	F (ATTGCCCTCTTCTGTCTGGCTACAC) R (AGACGAGGAGAAAACATCACAATCAC)	102	59
Am3	S13	XM_004243506.2	F (CAAACATGTGATTGGATAAAGAAACG) R (CTGACCAACCAAACTTTCCTGAT)	115	53
Am4	HsfA1a	NM_001309248.1	F (CGACCTCGACCCGAATAGTT) R (CGGAGGATCCCAAACCACAA)	262	55
Am5	P5CS	U60267.1	F (TGCACTGGAAGCAAATGAAA) R (CCATCAGCAATCTCCGTTCT)	207	54
Am6	APX	NM_001247853.2	F (TGTGATCCTGCTTTCCGTCC) R (ATCGTCTAACGTAGCTGCCA)	249	55

Table (2): The effect of pretreatment on shoot and root length, fresh and dry weight, leaves and branch number and leaf area of examined cultivars under stress and non-stress conditions.

Morphological Traits	Cultivars											
	Carmen (CAR)			Hybrid Super Strain B (HB)			Typhone (TY)			Agyad 16 (AG)		
	Pretreatment											
	Control	Heating	Cooling	Control	Heating	Cooling	Control	Heating	Cooling	Control	Heating	Cooling
Without stress (at green house temp.)												
Shoot length	$^{c}10.70^{gh}$	$^{b}13.90^{e}$	$^{a}25.30^{a}$	$^{b}14.00^{de}$	$^{c}12.00^{fg}$	$^{a}15.90^{c}$	$^{c}12.90^{ef}$	$^{b}16.00^{c}$	$^{a}21.00^{b}$	$^{a}13.90^{e}$	$^{a}13.70^{e}$	$^{a}15.30^{cd}$
Root length	$^{a}8.90^{de}$	$^{a}8.90^{de}$	$^{a}9.00^{de}$	$^{b}10.80^{c}$	$^{b}10.50^{c}$	$^{a}12.80^{ab}$	$^{a}11.50^{c}$	$^{a}11.70^{bc}$	$^{b}9.20^{d}$	$^{a}13.90^{a}$	$^{c}7.40^{fgh}$	$^{b}10.00^{c}$
Leaves number	$^{ab}21.50^{d}$	$^{b}19.00^{e}$	$^{a}24.00^{bc}$	$^{b}18.00^{e}$	$^{b}16.70^{ef}$	$^{a}21.70^{cd}$	$^{b}21.70^{cd}$	$^{c}16.70^{ef}$	$^{a}25.00^{b}$	$^{b}23.00^{bcd}$	$^{b}22.00^{cd}$	$^{a}28.70^{a}$
Leaf area	$^{a}5.48^{b}$	$^{a}4.20^{b-f}$	$^{a}5.00^{bc}$	$^{a}4.90^{bcd}$	$^{a}3.80^{c-g}$	$^{a}5.20^{bc}$	$^{a}8.00^{a}$	$^{b}4.20^{b-f}$	$^{b}5.60^{b}$	$^{a}8.20^{a}$	$^{b}3.80^{c-g}$	$^{a}7.20^{a}$
Branch number	$^{a}4.50^{abc}$	$^{a}4.30^{a-d}$	$^{a}5.30^{a}$	$^{a}4.70^{abc}$	$^{a}4.30^{a-d}$	$^{a}50^{ab}$	$^{a}4.30^{a-d}$	$^{a}3.70^{c-f}$	$^{a}4.70^{abc}$	$^{b}4.30^{a-d}$	$^{b}4.00^{b-e}$	$^{a}5.00^{ab}$
Fresh weigh	$^{a}4.40^{c}$	$^{b}3.10^{d}$	$^{a}4.60^{c}$	$^{b}2.50^{d-g}$	$^{b}3.20^{d}$	$^{a}5.50^{b}$	$^{b}2.70^{def}$	$^{b}2.60^{def}$	$^{a}4.50^{c}$	$^{b}4.65^{c}$	$^{c}2.93^{de}$	$^{a}8.20^{a}$
Dry weight	$^{a}0.53^{c}$	$^{b}0.34^{fgh}$	$^{a}0.54^{bc}$	$^{b}0.49^{cd}$	$^{c}0.37^{ef}$	$^{a}0.62^{b}$	$^{ab}0.44^{de}$	$^{b}0.32^{f}$	$^{a}0.53^{c}$	$^{b}0.47^{cd}$	$^{b}0.38^{ef}$	$^{a}0.91^{a}$
Under stress (at 7°C)												
Shoot length	$^{b}10.20^{hi}$	$^{a}12.10^{fg}$	$^{b}8.90^{ijk}$	$^{a}7.20^{l}$	$^{a}8.00^{kl}$	$^{a}7.50^{kl}$	$^{b}8.50^{jkl}$	$^{a}10.30^{h}$	$^{ab}10.00^{hi}$	$^{a}10.50^{h}$	$^{a}9.50^{hij}$	$^{b}7.30^{l}$
Root length	$^{c}6.60^{h}$	$^{b}8.50^{def}$	$^{a}11.30^{c}$	$^{a}7.30^{gh}$	$^{a}8.30^{d-g}$	$^{a}8.10^{d-g}$	$^{b}7.20^{gh}$	$^{a}8.80^{de}$	$^{a}8.80^{def}$	$^{a}7.80^{e-h}$	$^{a}8.20^{d-g}$	$^{a}7.50^{fgh}$
Leaves number	$^{ab}21.50^{d}$	$^{b}19.00^{e}$	$^{a}24.00^{bc}$	$^{b}18.00^{e}$	$^{b}16.70^{ef}$	$^{a}21.70^{cd}$	$^{b}21.70^{cd}$	$^{c}16.70^{ef}$	$^{a}25.00^{b}$	$^{b}23.00^{bcd}$	$^{b}22.00^{cd}$	$^{a}28.70^{a}$
Leaf area	$^{a}5.48^{b}$	$^{a}4.20^{b-f}$	$^{a}5.00^{bc}$	$^{a}4.90^{bcd}$	$^{a}3.80^{c-g}$	$^{b}5.60^{b}$	$^{a}8.00^{a}$	$^{b}4.20^{b-f}$	$^{b}5.60^{b}$	$^{a}8.20^{a}$	$^{b}3.80^{c-g}$	$^{a}7.20^{a}$
Branch number	$^{a}4.70^{abc}$	$^{a}4.30^{a-d}$	$^{a}5.30^{a}$	$^{a}4.50^{abc}$	$^{a}4.30^{a-d}$	$^{a}5.00^{ab}$	$^{b}4.30^{a-d}$	$^{a}3.70^{c-f}$	$^{a}4.70^{abc}$	$^{a}4.30^{a-d}$	$^{b}40^{b-e}$	$^{a}5.000^{ab}$
Fresh weigh	$^{a}1.71^{g-j}$	$^{a}2.13^{e-h}$	$^{a}1.60^{hij}$	$^{a}1.10^{j}$	$^{a}1.70^{g-j}$	$^{a}1.30^{ij}$	$^{a}1.90^{f-j}$	$^{a}2.10^{f-i}$	$^{a}1.70^{g-j}$	$^{a}2.10^{f-i}$	$^{ab}1.60^{hij}$	$^{b}1.40^{hij}$
Dry weight	$^{a}0.13^{gh}$	$^{a}0.13^{gh}$	$^{a}0.15^{gh}$	$^{a}0.12^{gh}$	$^{a}0.14^{gh}$	$^{a}0.10^{h}$	$^{a}0.16^{gh}$	$^{a}0.20^{g}$	$^{a}0.17^{gh}$	$^{a}0.18^{gh}$	$^{a}0.12^{gh}$	$^{a}0.13^{gh}$

Cultivars: CAR (Carmen); AG (Agyad16); HB (Hybrid super strain B); TY (Typhoon).

* The letters in the right indicated the interference effect of pretreatments, cultivars and stress or non-stress, while the letters on the left indicated the effect of pretreatment only under non-stress or stress conditio

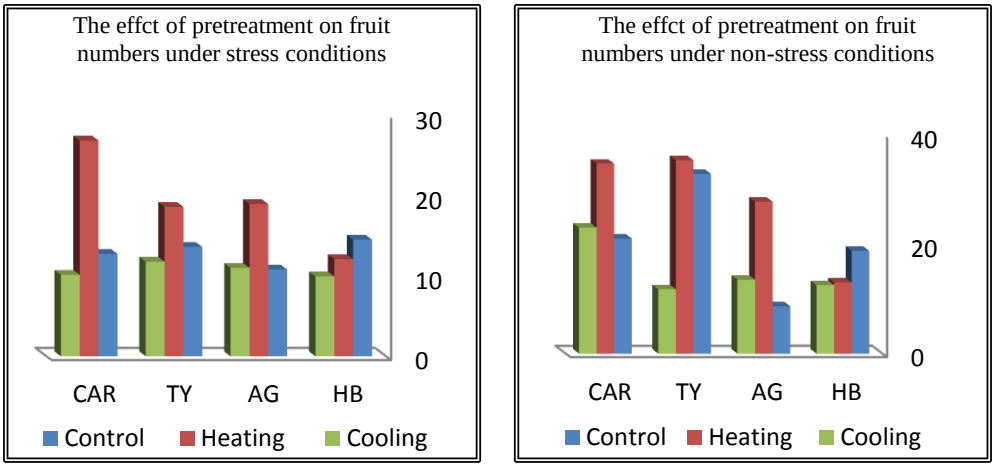

Fig. (1): The effect of pretreatment on fruit numbers in the examined cultivars in field stage under stress and non-stress conditions. CAR: Carmen; AG: Agyad16; TY: Typhoon; HB: Hybrid super strain B.

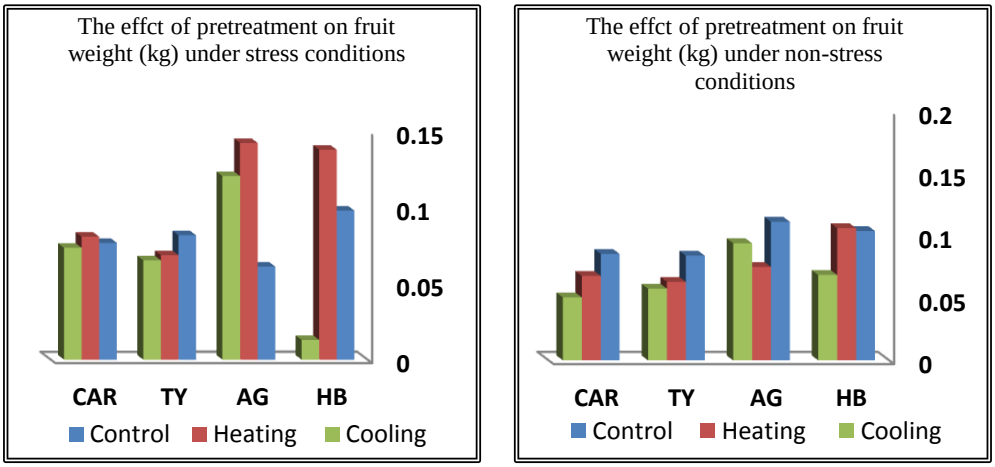

Fig. (2): The effect of pretreatment on fruit weight in the examined cultivars in field stage under stress and non-stress conditions. CAR: Carmen; AG: Agyad16; TY: Typhoon; HB: Hybrid super strain B.

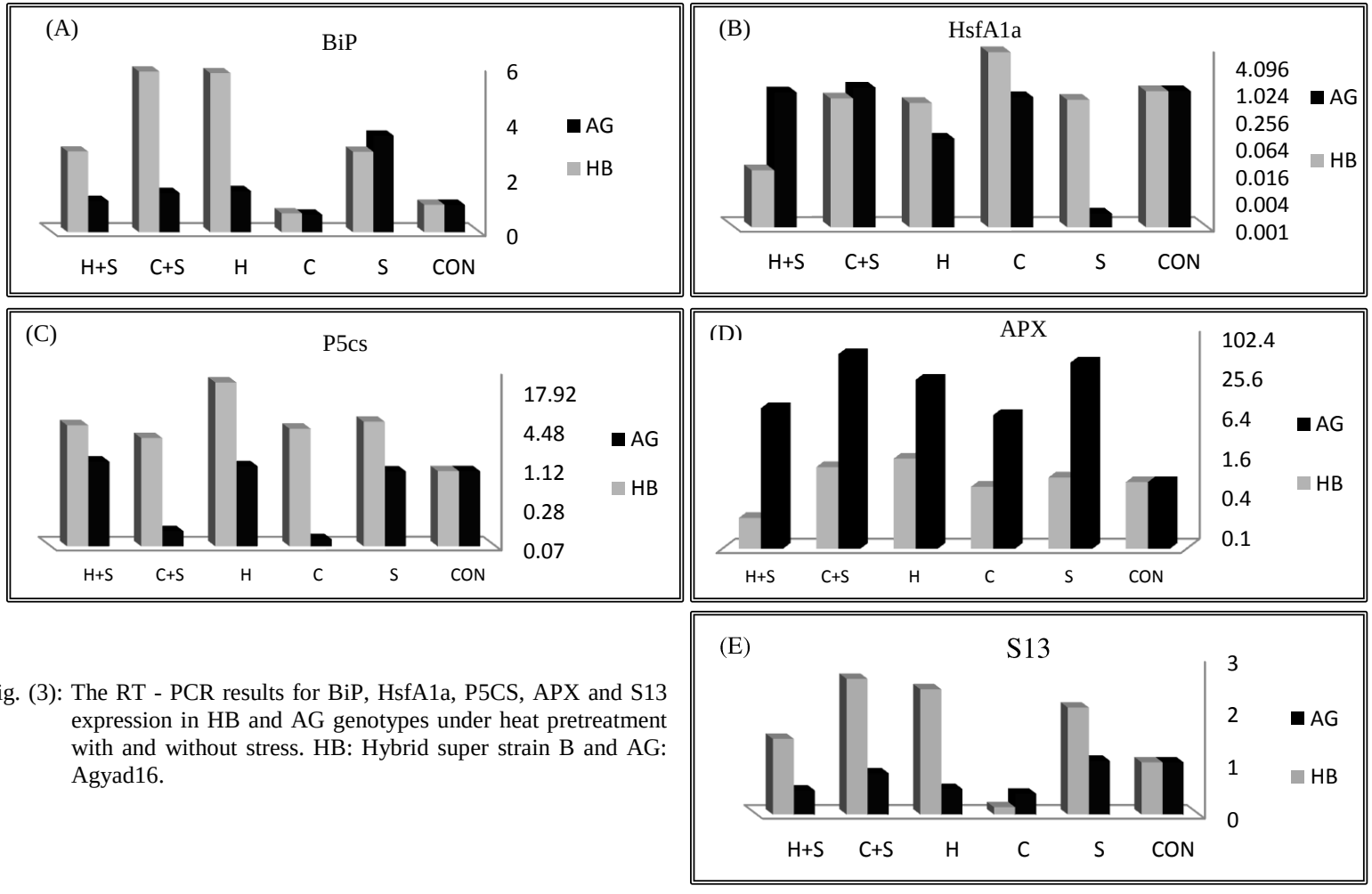

Fig. (3): The RT - PCR results for BiP, HsfA1a, P5CS, APX and S13 expression in HB and AG genotypes under heat pretreatment with and without stress. HB: Hybrid super strain B and AG: Agyad16.